

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61L 27/00	A1	(11) International Publication Number: WO 95/33502 (43) International Publication Date: 14 December 1995 (14.12.95)
(21) International Application Number: PCT/US95/06724 (22) International Filing Date: 2 June 1995 (02.06.95) (30) Priority Data: 253,398 3 June 1994 (03.06.94) US (71) Applicant: CREATIVE BIOMOLECULES, INC. [US/US]; 45 South Street, Hopkinton, MA 01748 (US). (72) Inventors: KHOURI, Roger, K.; Two Kingsbury Place, St. Louis, MO 63112 (US). SAMPATH, Kuber, T.; Six Spring Street, Medway, MA 02053 (US). RUEGER, David, C.; 19 Downey Street, Hopkinton, MA 01748 (US). (74) Agent: VITO, Christine, C.; Testa, Hurwitz & Thibault, High Street Tower, 125 High Street, Boston, MA 02110-2711 (US).		(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IS, JP, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: MANUFACTURE OF AUTOGENOUS REPLACEMENT BODY PARTS (57) Abstract <p>Disclosed are matrix materials, methods, and devices for manufacture <i>in vivo</i> of autogenous replacement body parts comprising plural distinct tissues. In one embodiment, the replacement body part is a skeletal joint and the new plural distinct tissues include bone and articular cartilage.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

WO 95/33502

PCT/US95/06724

MANUFACTURE OF AUTOGENOUS REPLACEMENT BODY PARTS

Relation to Related Applications

This application is a continuation-in-part of USSN 08/253,398, filed June 3, 1994, the disclosure of which is incorporated herein by reference.

5 Field of the Invention

This invention relates to materials and methods for the repair and regeneration of plural distinct tissues at a single defect site in a mammal. More particularly, the invention is concerned with materials and methods for the manufacture in vivo of autogenous replacement body parts, including mammalian skeletal joints, comprising plural different tissues, such as ligament, articular
10 cartilage and bone tissues.

Background of the Invention

Skeletal joints provide a movable union of two or more bones. Synovial joints are highly evolved articulating joints that permit free movement. Because mammalian lower limbs are concerned with locomotion and upper limbs provide versatility of movement, most of the joints in
15 the extremities are of the synovial type. There are various types of synovial joints. Their classification is based upon the types of active motion that they permit (uniaxial, biaxial, and polyaxial). They are differentiated further according to their principal morphological features (hinge, pivot, condyloid). In contrast to fibrous and cartilaginous joints where the ends of the bones are found in continuity with intervening tissue, the ends of the bones in a synovial joint are in
20 contact, but separate. Because the bones are not bound internally, the integrity of a synovial joint results from its ligaments and capsule (which bind the articulation externally) and to some extent from the surrounding muscles. In synovial joints, the contiguous bony surfaces are covered with articular or, hyaline cartilage, and the joint cavity is surrounded by a fibrous capsule which segregates the joint from the surrounding vascularized environment. The inner surface of the
25 capsule is lined by a synovial layer or "membrane" containing cells involved in secreting the viscous lubricating synovial fluid. Gray, Anatomy of the Human Body, pp. 312; 333-336 (13th ed.; C.C. Clemente, ed., (1985)).

WO 95/33502

PCT/US95/06724

In certain synovial joints, the joint or synovial cavity may be divided by a meniscus of fibrocartilage. Synovial joints involving two bones and containing a single joint cavity are referred to as simple joints. Joints that contain a meniscus forming two joint cavities are called composite joints. The term compound joint is used for those articulations in which more than a single pair of articulating surfaces are present.

Joint replacement, particularly articulating joint replacement, is a commonly performed procedure in orthopedic surgery. However, the ideal material for replacement joints remains elusive. Typically, joint reconstruction requires repair of the bony defect, the articular cartilage and, in addition, one or more of the joining ligaments. To date, there are no satisfactory clinical means for readily repairing both articular cartilage and bony defects within a joint which reliably results in viable, fully-functional weight-bearing joints. Prosthetic joints which replace all the endogenous joint tissues circumvent some of these problems. However, prosthetic joints have numerous, well documented limitations, particularly in younger and highly active patients. In addition, in some circumstances prosthetic joint replacement is not possible and repair options are limited to osteochondroallograft materials.

The articular, or hyaline cartilage, found at the end of articulating bones is a specialized, histologically distinct tissue and is responsible for the distribution of load resistance to compressive forces, and the smooth gliding that is part of joint function. Articular cartilage has little or no self-regenerative properties. Thus, if the articular cartilage is torn or worn down in thickness or otherwise damaged as a function of time, disease or trauma, its ability to protect the underlying bone surface is compromised.

Other types of cartilage in skeletal joints include fibrocartilage and elastic cartilage. Secondary cartilaginous joints are formed by discs of fibrocartilage which join vertebrae in the vertebral column. In fibrocartilage, the mucopoly-saccharide network is interlaced with prominent collagen bundles and the chondrocytes are more widely scattered than in hyaline cartilage. Elastic cartilage contains collagen fibers which are histologically similar to elastin fibers. As with other connective tissues the formation of cartilaginous tissue is a complex biological process, involving the interaction of cells and collagen fibers in a unique biochemical milieu.

Cartilage tissue, including articular cartilage, unlike other connective tissues, lacks blood vessels, nerves, lymphatics and basement membrane. Cartilage is composed of chondrocytes which

WO 95/33502

PCT/US95/06724

synthesize an abundant extracellular milieu composed of water, collagens, proteoglycans and noncollagenous proteins and lipids. Collagen serves to trap proteoglycans and to provide tensile strength to the tissue. Type II collagen is the predominant collagen in cartilage tissue. The proteoglycans are composed of a variable number of glycosaminoglycan chains, keratin sulphate, 5 chondroitin sulphate and/or dermatan sulphate, and N-linked and O-linked oligosaccharides covalently bound to a protein core. The sulfated glycosaminoglycans are negatively charged resulting in an osmotic swelling pressure that draws in water.

In contrast, certain collagens such as the fibrotic cartilaginous tissues which occur in scar tissue for example, are keloid and typical of scar-type tissue, i.e., composed of capillaries and 10 abundant, irregular, disorganized bundles of Type I and Type II collagen.

Histologically, articular or hyaline cartilage can be distinguished from other forms of cartilage, both by its morphology and by its biochemistry. Morphologically, articular cartilage is characterized by superficial versus mid versus deep "zones" which show a characteristic gradation of features from the surface of the tissue to the base of the tissue adjacent to the bone. In the 15 superficial zone, for example, chondrocytes are flattened and lie parallel to the surface embedded in an extracellular network that contains tangentially arranged collagen and few proteoglycans. In the mid zone, chondrocytes are spherical and surrounded by an extracellular network rich in proteoglycans and obliquely organized collagen fibers. In the deep zone, close to the bone, the collagen fibers are vertically oriented. The keratin sulphate rich proteoglycans increase in 20 concentration with increasing distance from the cartilage surface. For a detailed description of articular cartilage micro-structure, see, for example, (Aydelotte and Kuettner, (1988), Conn. Tiss. Res. 18:205; Zanetti et al., (1985), J. Cell Biol. 101:53; and Poole et al., (1984), J. Anat. 138:13.

Biochemically, articular collagen can be identified by the presence of Type II and Type IX collagen, as well as by the presence of well-characterized proteoglycans, and by the absence of 25 Type X collagen, which is associated with endochondral bone formation.

In normal articular cartilage, a balance exists between synthesis and destruction of the above-described extracellular network. However, in tissue subjected to repeated trauma, for example due to friction between misaligned bones in contact with one another, or in joint diseases characterized by net loss of articular cartilage, e.g., osteoarthritis, an imbalance occurs between 30 synthesis and degradation.

WO 95/33502

PCT/US95/06724

Two types of defects are recognized in articular surfaces, i.e., full-thickness defects and superficial defects. These defects differ not only in the extent of physical damage to the cartilage, but also in the nature of the repair response each type of lesion can elicit.

Full-thickness defects of an articulating surface include damage to the hyaline cartilage, the calcified cartilage layer and the subchondral bone tissue with its blood vessels and bone marrow. Full-thickness defects can cause severe pain since the bone plate contains sensory nerve endings. Such defects generally arise from severe trauma and/or during the late stages of degenerative joint disease, such as osteoarthritis. Full-thickness defects may, on occasion, lead to bleeding and the induction of a repair reaction from the subchondral bone. In such instances, however, the repair tissue formed is a vascularized fibrous type of cartilage with insufficient biomechanical properties, and does not persist on a long-term basis.

In contrast, superficial defects in the articular cartilage tissue are restricted to the cartilage tissue itself. Such defects are notorious because they do not heal and show no propensity for repair reactions. Superficial defects may appear as fissures, divots, or clefts in the surface of the cartilage, or they may have a "crab-meat" appearance in the affected tissue. They contain no bleeding vessels (blood spots) such as are seen in full-thickness defects. Superficial defects may have no known cause, however, they are often the result of mechanical derangements which lead to a wearing down of the cartilaginous tissue. Such mechanical derangements may be caused by trauma to the joint, e.g., a displacement of torn meniscus tissue into the joint, meniscectomy, a laxation of the joint by a torn ligament, malalignment of joints, or bone fracture, or by hereditary diseases. Superficial defects are also characteristic of early stages of degenerative joint diseases, such as osteoarthritis. Since the cartilage tissue is not innervated or vascularized, superficial defects do not heal and often degenerate into full-thickness defects.

Replacement with prosthetic joints is currently the preferred option for serious degeneration of joint function involving loss of articular cartilage. It is anticipated that a means for functional reconstruction of joint complexes, including regeneration and repair of articular cartilage, will have a profound effect on alloplastic joint replacement surgery and the management of degenerative joint disease.

Like articular cartilage, joint ligaments which serve to connect interacting bones in the joint, have little or no self-regenerative properties. Ligaments typically are composed of

WO 95/33502

PCT/US95/06724

substantially parallel bundles of white fibrous tissue. They are pliant and flexible to allow substantially complete freedom of movement, but are inextensible to prevent over-extension of the interacting bones in the joint. Like cartilage, ligament tissue is substantially devoid of blood vessels and has little or no self-regenerative properties. Surgical repair of torn or damaged

5 ligament tissue to date is limited to use of autogenous grafts or synthetic materials that are surgically attached to the articular extremities of the bones. Allogenic ligaments typically fail mechanically, presumably due to the treatments required to render these materials biocompatible. Similarly, tendons are rope-like structures which connect muscle fibers to bone or cartilage and which are formed from substantially parallel fibroids of white connective tissue. The synovial

10 capsule is composed of a thin layer of ligamentous tissue which encloses the joint and allows the joint to be bathed in the lubricating synovial fluid. The interior of the joint capsule is lined with a thin membrane of connective tissue having branched connective-tissue corpuscles defining the synovial membrane, and which is primarily responsible for secreting synovial fluid into the cavity. The integrity of this membrane therefore, is important to maintaining a source for the lubricating

15 synovial fluid. Repair of these tissues in orthopedic contexts typically is limited to resuturing of existing tissue.

Bone tissue differs significantly from the other tissues described hereinabove, including cartilage tissue. Specifically, bone tissue is vascularized tissue composed both of cells and a biphasic medium which is composed of a mineralized, inorganic component (primarily

20 hydroxyapatite crystals) and an organic component comprised primarily of Type I collagen. Glycosaminoglycans constitute less than 2% of this organic component and less than 1% of the biphasic medium itself or of bone tissue per se. Moreover, relative to cartilage tissue, the collagen present in bone tissue exists in a highly-organized parallel arrangement.

Bony defects, whether from degenerative, traumatic or cancerous etiologies, pose a

25 formidable challenge to the reconstructive surgeon. Particularly difficult is reconstruction or repair of skeletal parts that comprise part of a multi-tissue complex, such as occurs in mammalian joints.

Mammalian bone tissue is known to contain one or more proteinaceous materials presumably active during growth and natural bone healing which can induce a developmental cascade of cellular events resulting in endochondral bone formation. The developmental cascade

30 involved in endochondral bone differentiation consists of chemotaxis of mesenchymal cells,

WO 95/33502

PCT/US95/06724

proliferation of progenitor cells into chondrocytes and osteoblasts, differentiation of cartilage, vascular invasion, bone formation, remodeling, and finally marrow differentiation.

True osteogenic factors capable of inducing the above-described cascade of events that result in endochondral bone formation have now been identified, isolated, and cloned. These proteins, which occur in nature as disulfide-bonded dimeric proteins, are referred to in the art as "osteogenic" proteins, "osteoinductive" proteins, and "bone morphogenetic" proteins. Whether naturally-occurring or synthetically prepared, these osteogenic proteins, when implanted in a mammal typically in association with a substrate that allows the attachment, proliferation and differentiation of migratory progenitor cells, are capable of inducing recruitment of accessible progenitor cells and stimulating their proliferation, inducing differentiation into chondrocytes and osteoblasts, and further inducing differentiation of intermediate cartilage, vascularization, bone formation, remodeling, and finally marrow differentiation. Those proteins are referred to as members of the Vgr-1/OP1 protein subfamily of the TGF β super gene family of structurally related proteins. Members include the proteins described in the art as OP1 (BMP-7), OP2 (BMP-8), BMP2, BMP3, BMP4, BMP5, BMP6, 60A, DPP, Vgr-1 and Vgl. See., e.g., U.S. 5,011,691; U.S. 5,266,683, Ozkaynak et al. (1990) EMBO J. 2: 2085-2093, Wharton et al. (1991) PNAS 88:9214-9218), (Ozkaynak (1992) J. Biol. Chem. 267:25220-25227 and U.S. 5,266,683); (Celeste et al. (1991) PNAS 87:9843-9847); (Lyons et al. (1989) PNAS 86:4554-4558). These disclosures describe the amino acid and DNA sequences, as well as the chemical and physical characteristics of these proteins. See also (Wozney et al. (1988) Science 242:1528-1534); BMP 9 (WO93/00432, published January 7, 1993); DPP (Padgett et al. (1987) Nature 325:81-84; and Vg-1 (Weeks (1987) Cell 51:861-867).

It is an object of the instant invention to provide a bioresorbable matrix and device, suitable for regenerating body parts which comprise two or more functionally- and structurally-associated yet distinct replacement tissues in a mammal. Another object is to provide compositions and methods for the repair or complete reconstruction of a mechanically and functionally viable skeletal joint in a mammal, particularly an articulating or synovial joint, as well as other body parts comprising bone and bona fide hyaline cartilage, without relying on prosthetic devices. Another object is to provide materials and methods for the repair of tissue defects in an articulating mammalian joint, so as to form a mechanically and functionally viable joint comprising bone and articular cartilage, ligament, tendon, synovial membrane and synovial capsule tissue. Another

WO 95/33502

PCT/US95/06724

object of the invention is to provide means for restoring functional non-mineralized tissue in a skeletal joint including the avascular tissue therein.

Summary of the Invention

In accordance with the present invention, methods and devices are provided for the
5 manufacture of a live autogenous replacement part comprising plural distinct tissues. In one aspect the replacement body part includes part or all of a mammalian skeletal joint, including an articulating or synovial joint. As described herein below, the methods and compositions of the invention are sufficient to restore mechanical and functional viable of the tissues associated with a skeletal joint, including bone (and bone marrow), articular cartilage, ligament, tendon, synovial
10 capsule and synovial membrane tissues. Thus the invention provides methods and compositions for replacement of one or more of the plural distinct tissues that define a mammalian skeletal joint.

The invention provides, in one aspect therefore, a novel matrix for forming a mechanically and structurally functional, mammalian, replacement body part comprising plural distinct tissues. The matrix comprises intact residues specific for or characteristic of, and/or derived from at least
15 two distinct tissues of the replacement body part. As will be appreciated from the description provided herein below, the matrix can include residues specific for four or more distinct tissues. The matrix is biocompatible and bioresorbable. Specifically, it is sufficiently free of pathogens and antigenic stimuli that can result in graft rejection. Preferably the matrix is derived from an allogenic or xenogenic body part. Preferably, it is derived from a mammalian donor, such as a
20 cadaver. The body part may be rendered inert or "devitalized" by dehydration, such as by ethanol extraction and lyophilization, so that no residual cellular metabolism remains, but the function of endogenous growth factors and the like can be restored upon in situ reconstitution by endogenous body fluids. The treated body part which now is substantially depleted in antigenic and pathogenic components and now is biocompatible, maintains the residues specific for the plural distinct tissues
25 constituting the body part sought to be replaced. These residues include those of plural distinct tissues with dimensions and structural relationships to each other which mimic those of the body part to be replaced.

The thus treated matrix having utility in the methods and devices of the invention lacks significant mechanical integrity as compared with native tissue and, on its own, is not sufficient to
30 induce regeneration of a replacement body part or tissue when implanted. However, by

WO 95/33502

PCT/US95/06724

impregnating or otherwise infusing the interstices of the matrix with osteogenic protein so that the protein is disposed on or adsorbed to, the surfaces of the matrix, the device of the instant invention is formed and is sufficient to induce formation of new tissue in vivo such that regeneration of a mechanically and functionally viable replacement body part occurs in situ.

5 In one preferred embodiment, the device comprises part or all of a skeletal joint excised from a mammalian donor allogenic or xenogenic to the donee. Treated as described herein the device comprising the allogenic or xenogenic skeletal joint (1) is biocompatible, namely, it is non-pathogenic and sufficiently non-antigenic to prevent graft rejection in vivo, and (2) is sufficient to induce formation of a functionally viable autogenous replacement joint in vivo, including
10 generating functional bone, articular cartilage, ligament and capsule tissue in correct relation to one another such that a structurally and mechanically functional replacement joint results.

In another embodiment, the invention provides a device which serves as a template for forming in vivo part or all of a skeletal synovial joint comprising plural distinct tissues and which, in response to morphogenic signals, induces new tissue formation, including new articular cartilage
15 tissue from responding cells present in the synovial environment. The newly formed tissues assume the shape and function of the original tissue in the skeletal joint.

In another aspect, the invention provides methods for replacing a defective body part comprising the steps of: excising the defective body part and implanting the device of the instant invention. In one embodiment, the method also comprises the additional step of providing a supply
20 of mesenchymal cells to the implanted device, as by threading or otherwise providing a muscle flap perfused with a blood supply into a hollow portion of the device. In another embodiment, the device is implanted at a locus in the body of the individual distinct from the defect site but which allows generation of the replacement body part. The autogenous body part thus formed then can be implanted at the defect site.

25 As will be appreciated from the description provided herein, in another aspect, the invention provides devices and methods for the functional and mechanical restoration of one or more individual tissues in a mammalian skeletal joint, including the non-mineralized and avascular tissue therein. Thus, in one embodiment, the invention provides methods and devices competent for restoring, without limitation, functional articular cartilage, ligament, synovial membrane and
30 synovial capsule tissue. The methods and devices described herein can be used for example, to

WO 95/33502

PCT/US95/06724

correct superficial articular cartilage defects in a joint, to replace torn or compromised ligaments and/or tendons, and to repair defects in synovial capsule or membrane tissue.

5 The devices for repairing individual skeletal joint tissue comprise osteogenic protein disposed on a matrix containing residues specific for, or derived from skeletal joint tissue of the type to be restored, including, without limitation, cartilage, ligament, tendon, synovial capsule, or synovial membrane tissue. The device can take the form of a solid, or it can have the physical properties of a paste or gel. Preferably, the matrix is derived from allogenic or xenogenic tissue, and is treated as described herein to form a biocompatible devitalized matrix.

10 In another embodiment the matrix can be formulated de novo from synthetic and/or naturally-derived components. The matrix includes both (a) residues specific for, or characteristic of, the given tissue and, (b) materials sufficient to create a temporary scaffold for infiltrating cells and defining a three dimensional structure which mimics the dimensions of the desired replacement tissue. Useful such materials are described herein below. Suitable tissue-specific residues can be obtained from devitalized allogenic or xenogenic tissue and combined with the structural materials
15 as described herein to create the synthetic matrix. In another embodiment, the matrix comprises devitalized non-mineralized tissue. In some circumstances, as in the formation of articular cartilage on subchondral bone, a non-mineralized matrix material defining a three-dimensional structure which allows the attachment of infiltrating cells, can be sufficient, in combination with osteogenic protein, to induce new tissue formation.

20 While, as described above, in a preferred embodiment the invention contemplates a device suitable as a template for forming in vivo a replacement skeletal joint, as will be appreciated by the practitioner in the art, the invention contemplates, and the disclosure enables, a device suitable as a template for forming in vivo functional replacement body parts other than skeletal joints and which comprise plural distinct tissues.

25 When used in accordance with the methods of the instant invention, the devices of the invention and/or the tissues which result from their application, essentially satisfy the following criteria of a preferred grafting material:

1. They result in formation of mechanically and functionally viable tissues normally present at the site. These tissues are of an appropriate size and have correct structural

WO 95/33502

PCT/US95/06724

relationships so as to result in a functional body part. In particular, the multi-tissue replacement part, whether produced in situ at the site of intended use or remotely, becomes incorporated, integrating with adjacent tissues, essentially maintaining its shape, and avoiding abnormal resorption, regardless of the conditions present at the recipient site. Weiland et al. (1983) Clin.

5 Orthop. 174:87 (1983).

2. The devices are capable of being precisely contoured and shaped to exactly match any defect, whichever complex skeletal or organ shape it is meant to replace.

3. The devices virtually have unlimited supply and are relatively easy to obtain.

4. The devices have minimal donor site morbidity.

10 Furthermore, the instant invention provides practitioners with materials and methods for skeletal joint repair including the repair of the bone and articular cartilage present therein, and which solve problems that occur using the methods and devices of the art. For example, the instant invention can induce formation of bona fide hyaline cartilage rather than fibrocartilage at a defect site. Using the materials and methods disclosed herein, functional hyaline cartilage forms on the

15 articulating surface of bone at a defect site and does not degenerate over time to fibro-cartilage. By contrast, prior art methods of repairing cartilage defects generally ultimately result in development of fibrous cartilage at the defect site. Unlike hyaline cartilage, fibrocartilage lacks the physiological ability to restore articulating joints to their full capacity. Thus, when the instant materials are used in accordance with the instant methods, the practitioner can substantially

20 functionally restore a cartilage defect in an articulating joint, particularly a superficial articular cartilage defect and substantially avoid the undesirable formation of fibrocartilage typical of prior art methods, or degeneration into a "full-thickness defect". The invention also provides means for repairing individual tissue of a joint not readily repairable individually using prior art methods, and which, in some cases, previously warranted replacement of the entire joint with a prosthetic device.

25 The invention further allows use of allogenic replacement materials for repairing the avascular tissue in a skeletal joint, and which result in the formation of mechanically and functionally viable replacement tissues at a joint locus.

WO 95/33502

PCT/US95/06724

Brief Description of the Drawings

While the specification concludes with claims particularly pointing out and specifically claiming the subject matter which is regarded as constituting the present invention, it is believed that the invention will be better understood from the following detailed description of preferred
5 embodiments taken in conjunction with the accompanying drawings in which:

Fig. 1 is a fragmentary front elevational view of a mammalian knee joint with sufficient tissue removed to show the articular cartilage on the condyles of the femur, the ligaments, synovial membrane, joint capsule, and further showing a damaged area in the articular cartilage requiring repair;

10 FIGS. 2A through 2D are schematic representations of the elements used to generate a viable, functional glenohumoral hemi-joint in one embodiment of the invention. Fig. 2A depicts a lyophilized allograft; Fig. 2B depicts osteogenic protein for application to the lyophilized allograft of Fig. 2A; Fig. 2C depicts a muscle flap of cutaneous maximus muscle to be threaded inside the shaft of the lyophilized allograft; and, Fig. 2D depicts a viable, functional hemi-joint resulting from
15 the combination of elements in Figs. 2A, 2B and 2C. Fig. 2D represents one embodiment of the device of the instant invention;

FIGS. 3A through 3D are schematic representations of the four allografts tested in the hemi-joint of Example 2 (5 week); and

FIGS. 4A through 4D are schematic representations of the four allografts tested in the
20 hemi-joint of Example 3 (6 month).

Detailed Description

In accordance with the present invention, novel materials and methods are provided for the repair and regeneration of plural distinct tissues, including manufacture of a live autogenous replacement part comprising plural distinct tissues. In one embodiment the replacement body part
25 is a skeletal joint, particularly an articulating joint, and includes, without limitation, residues specific for, or derived from, bone, cartilage, ligament, tendon, synovial capsule and synovial membrane tissue.

WO 95/33502

PCT/US95/06724

More particularly, in one aspect, the invention provides a device comprising an osteogenic protein disposed on the surfaces of a matrix or substrate for forming a functional, mammalian replacement body part comprising plural distinct tissues. As used herein, the term "matrix" is understood to define a structure having interstices for the attachment, proliferation and
5 differentiation of infiltrating cells. It comprises residues specific for the tissue to be replaced and/or derived from the same tissue type, and has a shape and dimension when implanted which substantially mimics that of the replacement tissue desired.

As used herein, the term "residue" is intended to mean a constituent of a given tissue, which has specificity for, or is characteristic of, the given tissue, and which is derivable from the
10 non-viable constituents of the given tissue. A matrix comprising these residue(s), when combined with osteogenic protein, and implanted in a mammal in an environment which mimics the tissue's local environment under physiological conditions, and is sufficient for formation of specific, mechanically and functionally viable replacement tissue.

The term "plural distinct tissue" is intended to mean physiologically distinguishable
15 tissues, such as biochemically or ultrastructurally distinguishable tissues which reside at an anatomically similar locus. In an articulating replacement joint device for example, the matrix can comprise residues specific for, or derived from, bone, cartilage, ligament, tendon and synovial membrane tissue. Thus, a significant aspect of the matrix of the invention is a single structure comprising residues of plural, distinct tissues, and which, when combined with an osteogenic
20 protein as defined herein, is suitable for inducing repair or regeneration of a body part that is mechanically and functionally viable over time in vivo.

As used herein, the terms "bone" and "articular cartilage" are intended to mean the following: Bone refers to a calcified (mineralized) connective tissue primarily comprising a composite of deposited calcium and phosphate in the form of hydroxyapatite, collagen
25 (predominantly Type I collagen) and bone cells, such as osteoblasts, osteocytes and osteoclasts, as well as to the bone marrow tissue which forms in the interior of true endochondral bone. Cartilage refers to a type of connective tissue that contains chondrocytes embedded in an extracellular network comprising fibrils of collagen (predominantly Type II collagen along with other minor types, e.g. Types IX and XI), various proteoglycans (e.g., chondroitin sulfate, keratan sulfate, and
30 dermatan sulfate proteoglycans), other proteins, and water. Articular cartilage refers to hyaline or articular cartilage, an avascular, non-mineralized tissue which covers the articulating surfaces of

WO 95/33502

PCT/US95/06724

the portions of bones in joints and allows movement in joints without direct bone-to-bone contact, and thereby prevents wearing down and damage to opposing bone surfaces. Most normal healthy articular cartilage is referred to as "hyaline," i.e., having a characteristic frosted glass appearance. Under physiological conditions, articular cartilage tissue rests on the underlying, mineralized bone surface, the subchondral bone, which contains highly vascularized ossicles. These highly vascularized ossicles can provide diffusible nutrients to the overlying cartilage, but not mesenchymal stem cells.

"Ligament" is intended to mean both the rope-like structures of white fibrous connective tissue which attach anterior extremities of interacting bones, as well as the tissue defining a synovial capsule. "Synovial membrane" is intended to define the connective tissue membrane lining the interior of the synovial cavity and which is involved in synovial fluid secretion. "Tendon" is intended to define the connective tissue structure which joins muscle to bone.

Replacement Body Parts

As disclosed herein, the instant invention provide methods and compositions for replacing and repairing a defective body part. The method comprises the steps of surgically excising the defective body part, implanting a device comprising a matrix of the type described above at the site of excision, and, as necessary, surgically repairing tissues adjacent the site of excision as described herein below. For example, for synovial joint replacement, it is desirable to repair the joint capsule, including the synovial membrane and ligaments, so as to surgically approximate the joint structure as it occurs physiological conditions, thereby recreating the avascular environment which is the synovial cavity and which is bathed in synovial fluid. It also is preferable to suture or otherwise mechanically temporarily connect the implanted device to surrounding tissue.

In one embodiment the device is constructed to replace part or all of a mammalian skeletal joint structure and includes a matrix having residues for plural, distinct tissues, including two or more of bone, cartilage, ligament, tendon, synovial capsule and/or synovial membrane tissue.

In another embodiment the device is constructed to replace an individual tissue of a mammalian skeletal joint, including an individual avascular and/or non-mineralized tissue. As demonstrated herein, the device is competent to induce functional replacement tissue formation, including articular cartilage, from responding cells present in the local environment, including a

WO 95/33502

PCT/US95/06724

synovial environment, and without requiring cellular infiltration of mesenchymal cells from a vascularized muscle flap. The matrix of this embodiment comprises residues specific for, or characteristic of, and/or derived from, tissue of the same type as the individual tissue to be replaced. In another embodiment, the matrix comprises devitalized non-mineralized tissue. In a preferred embodiment, the replacement tissue can include articular cartilage, ligament, bone, tendon or synovial capsule tissue.

In a partial or complete joint replacement, it is preferred but not required to include in the practice of the method the additional step of threading a muscle flap into a hollow portion of the implanted device. For example, using the method described in Khouri, U.S. 5,067,963, the disclosure of which is incorporated herein by reference and herewith below, a muscle flap, which can itself be pretreated with osteogenic protein, can be surgically introduced into a cavity in the implanted matrix, such as the marrow cavity of devitalized bone, to provide a blood supply to expedite morphogenesis of vascularized tissue and to provide a ready supply of mesenchymal stem cells.

The matrix of instant invention has utility as an implantable device when osteogenic protein is disposed on the surfaces of the matrix, present in an amount sufficient to induce formation of each of the replacement tissues. This permits regeneration of the body part within the mammal, including plural tissues of appropriate size, interrelationship, and function. Osteogenic proteins contemplated to be useful in the instant invention are described below and have been earlier-described in, for example, U.S. Pat. Nos. 4,968,550, 5,258,499 and 5,266,683, the disclosures of which are incorporated by reference herein. The osteogenic protein can be, for example, any of the known bone morphogenetic proteins and/or equivalents thereof described herein and/or in the art and includes naturally sourced material, recombinant material, and any material otherwise produced which is capable of inducing tissue morphogenesis.

The methods and materials of the instant invention are especially useful for the repair and/or partial or complete replacement of mammalian body joints, including, without limitation, articulating joints, particularly joints enclosed by a ligamentous capsule and bathed in synovial fluid.

In some synovial joints, the movement is uniaxial, i.e., all movements take place around one axis: Among these are the ginglymus or hinge joint in which the axis of movement is

WO 95/33502

PCT/US95/06724

transverse to the axes of the bones, and the trochoid or pivot joint in which the axis is longitudinal. In the case of biaxial synovial joints, movements are around two axes at a right angle or any other angle to each other: These include the condyloid, the ellipsoid, and the saddle joints. There is a third type of synovial joint, the spheroidal or ball-and-socket joint, in which the movements are polyaxial, i.e., movements are permitted in an infinite number of axes. Finally, there are the plane or gliding-type synovial joints.

In hinge joints, the articular surfaces are molded to each other in such a manner as to permit motion in only one plane around the transverse axis. Flexion at the elbow joint is an example; other examples include the interphalangeal joints of both the fingers and toes. In pivot joints, movement in a pivot joint also occurs around a single axis, however, it is the longitudinal axis. There are several pivot joints in the human body, such as the proximal radioulnar articulation. In condylar joints include, movement occurs principally in one plane. The tibiofemoral articulation of the knee joint is an example. In ellipsoid joint include, movement is around two principal axes which are at right angles to each other. Examples of these joints include the radiocarpal and metacarpophalangeal joints. In a saddle joint, the articular end of the proximal bone is concave in one axis and convex in a perpendicular axis. These surfaces fit reciprocally into convex and concave surfaces of the distal bone. The best example of a saddle joint is the carpometacarpal joint of the thumb. A ball-and-socket joint is one in which the distal bone is capable of motion around an indefinite number of axes with one common center. Examples of this form of articulation are found in the hip and shoulder joints. A plane or gliding-type joint allows a slight slipping or sliding of one bone over the other. Unlike the above-described joints, the amount of motion between the surfaces is limited by the ligaments or osseous processes that surround the articulation. This is the form present in the joints between the articular processes of certain vertebrae, the carpal joints, and the intermetatarsal joints.

Although it is contemplated that the present invention is usable to repair defects including bone and articular cartilage elsewhere in a mammalian body, aspects of the invention are here illustrated in connection with the articulating surfaces on the femur in a knee joint 10 illustrated in FIG. 1.

FIG. 1 illustrates a knee joint 10 between the bottom of a femur 11 and the top of a tibia 12. For clarity of illustration, only portions 13 and 14 of the medial and lateral collateral ligaments which movably tie the femur 11 to the underlying tibia 12 and fibula 15, are shown in

WO 95/33502

PCT/US95/06724

FIG. 1. Similarly, the joint capsule is represented by the exterior dark lining 25, and the synovial membrane, which lines the synovial cavity and secretes the lubricating synovial fluid, is represented by the interior dark lining 26. Normally interposed between the opposing surfaces of the femur 11 and tibia 12 are lateral and medial meniscus cartilages 16 and 17 and anterior and posterior cruciate ligaments (not shown). The convexly curved condyles 20 and 21 at the lower end of the femur 11 are normally supported by the meniscus cartilages 16 and 17, respectively, on the upper end of the tibia 12. Normally, the lower end of the femur 11, including the condyles 20 and 21, are covered by a layer 22 of hyaline cartilage material, referred to as the articular cartilage 22. The articular cartilage 22 forms a generally resilient padding which is fixed on the surface of the lower end of the femur 11 to protect the latter from wear and mechanical shock. Moreover, the articular cartilage 22, when lubricated by the synovial fluid in the knee joint 10, provides a surface which is readily slidable on the underlying surfaces of the meniscus cartilages 16 and 17 (or on the upper surface of the tibia 12 should one or both of the meniscus cartilage 16 and 17 be partly or totally absent) during articulation of the knee joint 10.

A portion of the articular cartilage may become damaged by injury or disease, or become excessively worn. FIG. 1 illustrates an example of a damaged area 23.

Matrix Considerations

As will be appreciated by the skilled artisan, provided the matrix has a three dimensional structure sufficient to act as a scaffold for infiltrating cells, and includes the residues specific for, or characteristic of, and/or which are derived from, the same tissue type as the tissue to be repaired, the precise nature of the substrate per se used for the matrices disclosed herein is not determinative of a matrix's ultimate ability to repair and regenerate replacement tissue. In the instant invention, the substrate serves as a scaffold upon which certain cellular events, mediated by an osteogenic protein, necessarily will occur. The specific responses to the osteogenic protein ultimately are dictated by the endogenous microenvironment at the implant site and the developmental potential of the responding cells. As also will be appreciated by the skilled artisan, the precise choice of substrate utilized for the matrices disclosed herein will depend, in part, upon the type of defect to be repaired, anatomical considerations such as the extent of vascularization at the defect site, and the like.

WO 95/33502

PCT/US95/06724

The matrix of the invention may be obtained as follows. A replacement tissue or body part to be used as a replacement body part and which comprises at least two distinct tissues in association to form the body part, is provided, as from a cadaver, or from a bone bank and treated, as by ethanol treatment and dehydrated by lyophilization, so that the remaining material is non-pathogenic and sufficient non-antigenic to prevent graft rejection. As described above, the thus treated material having utility in the devices of the invention further comprises the residues of the extracted tissue or tissues from which it is derived. A replacement body part matrix thus treated further is dimensioned such that the residues have a structural relationship to each other which mimic that of the body part to be replaced.

10 Natural-sourced Matrices

Suitable allogenic or xenogenic matrices can be created as described herein below, using methods well known in the art. Preferably, the replacement body part or tissue is obtained fresh, from a cadaver or from a tissue bank which freezes its tissues upon harvest. In all cases and as will be appreciated by the practitioner in the field, it is preferable to freeze any tissue upon harvest, unless the tissue is to be put to immediate use. Prior to use, the tissue is treated with a suitable agent to extract the cellular non-structural components of the tissue so as to devitalize the tissue. The agent also should be capable of extracting any growth inhibiting components associated with the tissue, as well as to extract or otherwise destroy any pathogens. The resulting material is an acellular matrix defining interstices that can be infiltrated by cells, and is substantially depleted in non-structurally-associated components.

In a currently preferred procedure, the tissue is devitalized following a methodology such as that used in the art for fixing tissue. The tissue is exposed to a non-polar solvent, such as 100% (200 proof) ethanol, for a time sufficient to substantially replace the water content of the tissue with ethanol and to destroy the cellular structure of the tissue. Typically, the tissue is exposed to 200 proof ethanol for several days, at a temperature in the range of about 4° - 40°C, taking care to replace the solution with fresh ethanol every 6-12 hours, until such time as the liquid content of the tissue comprises 70-90% ethanol. Typically, treatment for 3-4 days is appropriate. The volume of liquid added should be more than enough to submerge the tissue. The treated tissue then is lyophilized. The resulting, dry matrix is substantially depleted in non-structural components but retains both intracellular and extracellular matrix components derived from the tissue.

WO 95/33502

PCT/US95/06724

Numerous other methods are described in the art for extracting tissues, including mineralized tissue such as bone, and for rendering these tissues biocompatible for allogenic or xenogenic implants. See, for example, Sampath et al. (1983) PNAS 80:6591-6595, US 5,011,691, and U.S. Patent Nos. 4,975,526 and 5,171,574. These publications describe extraction with 4M
5 guanidine-HCl, 50mM Tris-HCl, pH 7.0 for 16 hours at 4°C, and various deglycosylating and collagen fibril modifying agents, including hydrogen fluoride, trifluoroacetic acid, dichloromethane, acetonitrile, isopropanol, heated, acidic aqueous solutions, and various combinations of these reagents. The disclosures of the patents is incorporated herein by reference. As described therein and below, where the matrix is treated with a fibril-modifying agent, the treated matrix can be
10 washed to remove any extracted components, following a form of the procedure set forth below:

1. Suspend matrix preparation in TBS (Tris-buffered saline) 1g/200 ml and stir at 4°C for 2 hrs; or in 6 M urea, 50 mM Tris-HCl, 500 mM NaCl, pH 7.0 (UTBS) or water and stir at room temperature (RT) for 30 minutes (sufficient time to neutralize the pH);
- 15 2. Centrifuge and repeat wash step; and
3. Centrifuge; discard supernatant; water wash residue; and then lyophilize.

Treated allogenic or xenogenic matrices are envisioned to have particular utility for creating devices for forming replacement body parts comprising plural distinct tissues, as well as for creating devices for replacing individual joint tissues, such as ligament and articular cartilage
20 tissue. For example, a replacement ligament device can be formulated from an allogenic ligament matrix and osteogenic protein, and implanted at a skeletal joint locus following standard surgical procedures for autogenous ligament replacement. Similarly, an allogenic articular cartilage device can be formed from devitalized cartilage tissue, or other inert, non-mineralized matrix material and osteogenic protein, and the device laid on the subchondral bone surface as a sheet. Alternatively, a
25 formulated device can be pulverized or otherwise mechanically abraded to produce particles which can be formulated into a paste or gel as described herein for application to the bone surface.

WO 95/33502

PCT/US95/06724

Synthetic Matrices

As an alternative to a natural-sourced matrix, or as a supplement to be used in combination with a natural-sourced matrix, a suitable matrix also can be formulated de novo, using (1) residues derived from and/or characteristic of, or specific for, the same tissue type as the tissue to be repaired, and (2) one or more materials which serve to create a three-dimensional scaffolding structure that can be formed or molded to take on the dimensions of the replacement tissue desired. In some circumstances, as in the formation of articular cartilage on a subchondral bone surface, osteogenic protein in combination with a matrix defining a three-dimensional scaffolding structure sufficient to allow the attachment of infiltrating cells and composed of a non-mineralized material can be sufficient. Any one or combination of materials can be used to advantage, including, without limitation, collagen; homopolymers or copolymers of glycolic acid, lactic acid, and butyric acid, including derivatives thereof; and ceramics, such as hydroxyapatite, tricalcium phosphate and other calcium phosphates and combinations thereof.

The tissue-specific component of a synthetic matrix readily can be obtained by devitalizing an allogenic or xenogenic tissue as described above and then pulverizing or otherwise mechanically breaking down the insoluble matrix remaining. This particulate material then can be combined with one or more structural materials, including those described herein. Alternatively, tissue-specific components can be further purified from the treated matrix using standard extraction procedures well characterized in the art and, using standard analysis procedures, the extracted material at each purification step can be tested for its tissue-specificity capability. See, for example, Sampath et al. (1987) PNAS 78:7599-7603 and US 4,968,590 for exemplary tissue extraction protocols.

A synthetic matrix may be desired where, for example, replacement articular cartilage is desired in an existing joint to, for example, correct a tear or limited superficial defect in the tissue, or to increase the height of the articular cartilage surface now worn due to age, disease or trauma. Such "resurfacing" of the articular cartilage layer can be achieved using the methods and compositions of the invention by, in one embodiment, treating a sheet of allogenic or xenogenic articular cartilage tissue as described herein, coating the resulting matrix with osteogenic protein, rolling up the formulated device so that it can be introduced to the joint using standard orthoscopic surgical techniques and, once provided to the site, unrolling the device as a layer onto the articular bone surface. In another embodiment, the device is formulated as a paste or injectable gel-like

WO 95/33502

PCT/US95/06724

substance that can be injected onto the articular bone surface in the joint also using standard orthoscopic surgical techniques. In this embodiment, the formulation may comprise a pulverized or otherwise mechanically degraded device comprising both matrix and osteogenic protein and, in addition, one or more components which serve to bind the particles into a paste-like or gel-like substance. Binding materials well characterized in the art include, for example, carboxymethylcellulose, glycerol, polyethylene-glycol and the like. Alternatively, the device can comprise osteogenic protein dispersed in a synthetic matrix which provides the desired physical properties. As an example, a synthetic matrix having tissue specificity for cartilage and bone is described in WO91/18558, published December 21, 1991 and herein below. Briefly, the matrix comprises a porous crosslinked structural polymer of biocompatible, biodegradable collagen and appropriate, tissue-specific glycosaminoglycans as tissue-specific cell attachment factors. Collagen derived from a number of sources can be used, including insoluble collagen, acid-soluble collagen, collagen soluble in neutral or basic aqueous solutions, as well as those collagens which are commercially available.

Glycosaminoglycans (GAGs) or mucopolysaccharides are hexosamine-containing polysaccharides of animal origin that have a tissue specific distribution, and therefore may be used to help determine the tissue specificity of the morphogen-stimulated differentiating cells. Reaction with the GAGs also provides collagen with another valuable property, i.e., inability to provoke an immune reaction (foreign body reaction) from an animal host.

Chemically, GAGs are made up of residues of hexoamines glycosidically bound and alternating in a more-or-less regular manner with either hexouronic acid or hexose moieties (see, e.g., Dodgson et al. in Carbohydrate Metabolism and its Disorders (Dickens et al., eds.) Vol. 1, Academic Press (1968)). Useful GAGs include hyaluronic acid, heparin, heparin sulfate, chondroitin 6-sulfate, chondroitin 4-sulfate, dermatan sulfate, and keratin sulfate. Other GAGs also can be used for forming the matrix described herein, and those skilled in the art will either know or be able to ascertain other suitable GAGs using no more than routine experimentation. For a more detailed description of mucopolysaccharides, see Aspinall, Polysaccharides, Pergamon Press, Oxford (1970).

Collagen can be reacted with a GAG in aqueous acidic solutions, preferably in diluted acetic acid solutions. By adding the GAG dropwise into the aqueous collagen dispersion, coprecipitates of tangled collagen fibrils coated with GAG results. This tangled mass of fibers

WO 95/33502

PCT/US95/06724

then can be homogenized to form a homogeneous dispersion of fine fibers and then filtered and dried.

Insolubility of the collagen-GAG products can be raised to the desired degree by covalently cross-linking these materials, which also serves to raise the resistance to resorption of these materials. In general, any covalent cross-linking method suitable for cross-linking collagen also is suitable for cross-linking these composite materials, although crosslinking by a dehydrothermal process is preferred.

Formulation Considerations

The devices of the invention can be formulated using any of the methods described in the art for formulating osteogenic devices. See, for example, US Patent No. 5,266,683, the disclosure of which is incorporated herein by reference. Briefly, osteogenic protein typically is dissolved in a suitable solvent and combined with the matrix. The components are allowed to associate. Typically, the combined material then is lyophilized, with the result that the osteogenic protein is disposed on, or adsorbed to the surfaces of the matrix. Useful solubilizing solvents include, without limitation, an ethanoltrifluoroacetic acid solution, e.g., 47.5% EtOH/0.01% TFA; and acetonitrile/TFA solution, ethanol or ethanol in water, and physiologically buffered saline solutions. Formulations in an acidic buffer can facilitate adsorption of OP1 onto the matrix surface. For the replacement body part devices of the invention, the currently preferred formulation protocol is incubation of matrix and osteogenic protein in an ethanol/TFA solution (e.g., 30-40% EtOH/0.01-0.1% TFA) for 24 hours, followed by lyophilization. This procedure is sufficient to adsorb or precipitate 70-90% of the protein onto the matrix surface.

The quantity of osteogenic protein used will depend on the size of replacement device to be used and on the specific activity of the osteogenic protein. Typically, 0.5 mg-100 mg/10 g of matrix, dry weight, can be used to advantage.

In addition to osteogenic proteins, various growth factors, hormones, enzymes, therapeutic compositions, antibiotics, or other bioactive agents also can be adsorbed onto, or impregnated within, a substrate and released over time when implanted and the matrix slowly is absorbed. Thus, various known growth factors such as EGF, PDGF, IGF, FGF, TGF- α , and TGF- β can be

WO 95/33502

PCT/US95/06724

released in vivo. The matrix can also be used to release chemotherapeutic agents, insulin, enzymes, enzyme inhibitors or chemotactic-chemoattractant factors.

Protein Considerations

As defined herein, the osteogenic proteins useful in the composition and methods of the invention include the family of dimeric proteins having endochondral bone activity when implanted in a mammal in association with a matrix and which comprise a subclass of the "super family" of "TGF β -like" proteins. The natural-sourced osteogenic protein in its mature, native form is a glycosylated dimer typically having an apparent molecular weight of about 30-36 kDa as determined by SDS-PAGE. When reduced, the 30 kDa protein gives rise to two glycosylated peptide subunits having apparent molecular weights of about 16 kDa and 18 kDa. In the reduced state, the protein has no detectable osteogenic activity. The unglycosylated protein, which also has osteogenic activity, has an apparent molecular weight of about 27 kDa. When reduced, the 27 kDa protein gives rise to two unglycosylated polypeptides having molecular weights of about 14 kDa to 16 kDa capable of inducing endochondral bone formation in a mammal. Useful sequences include those comprising the C-terminal 102 amino acid sequences of DPP (from *Drosophila*), Vgl (from *Xenopus*), Vgr-1 (from mouse), the OP1 and OP2 proteins, proteins (see U.S. Pat. No. 5,011,691 and Oppermann et al., as well as the proteins referred to as BMP2, BMP3, BMP4 (see WO88/00205, U. S. Patent No. 5,013,649 and W091/18098), BMP5 and BMP6 (see WO90/11366, PCT/US90/01630 and BMP8 and 9.

The members of this family of proteins share a conserved six or seven cysteine skeleton in the C-terminal region. See, for example, 335-431 of Seq. ID No. 1 and whose sequence defines the six cysteine skeleton residues referred to herein as "OPS", or residues 330-431 of Seq. ID No. 1, comprising 102 amino acids and whose sequence defines the seven cysteine skeleton.

This family of proteins includes longer forms of a given protein, as well as phylogenetic, e.g., species and allelic variants and biosynthetic mutants, including addition and deletion mutants and variants, such as those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration still allows the protein to form a dimeric species having a conformation capable of inducing bone formation in a mammal when implanted in the mammal in association with a matrix. In addition, the osteogenic proteins useful in devices of this invention may include forms having varying glycosylation patterns and varying N-termini, may be naturally occurring or

WO 95/33502

PCT/US95/06724

biosynthetically derived, and may be produced by expression of recombinant DNA in procaryotic or eucaryotic host cells. The proteins are active as a single species (e.g., as homodimers), or combined as a mixed species, including heterodimers.

In one embodiment, the osteogenic protein contemplated herein comprises OP1 or an OP1-
 5 related sequence. Useful OP1 sequences are recited in US Pat Nos. 5,011,691; 5,018,753 and
 5,266,683; in Ozkaynak et al. (1990) EMBO J 9:2085-2093; and Sampath et al. (1993) PNAS 90:
 6004-6008. OP-1 related sequences include xenogenic homologs, e.g.; 60A, from *Drosophila*,
 Wharton et al. (1991) PNAS 88:9214-9218; and proteins sharing greater than 60% identity with
 OP1 in the C-terminal seven cysteine domain, preferably at least 65% identity. Examples of OP-1
 10 related sequences include BMP5, BMP6 (and its species homolog Vgr-1, Lyons et al. (1989)
PNAS 86:4554-4558), Celeste, et al. (1990) PNAS 87:9843-9847 and PCT international
 application WO93/00432; OP-2 (Ozkaynak et al. (1992) J.Biol. Chem. 267:13198-13205) As will
 be appreciated by those having ordinary skill in the art, chimeric constructs readily can be created
 using standard molecular biology and mutagenesis techniques combining various portions of
 15 different morphogenic protein sequences to create a novel sequence, and these forms of the protein
 also are contemplated herein.

In another preferred aspect, the invention contemplates osteogenic proteins comprising
 species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX"
 which accommodates the homologies between the various identified species of the osteogenic OP1
 20 and OP2 proteins, and which is described by the amino acid sequence presented below and in
 Sequence ID No. 3.

Cys Xaa Xaa His Glu Leu Tyr Val Ser Phe

1 5 10

Xaa Asp Leu Gly Trp Xaa Asp Trp Xaa Ile

25 15 20

Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys

25 30

WO 95/33502

PCT/US95/06724

Glu Gly Glu Cys Xaa Phe Pro Leu Xaa Ser
 35 40
 Xaa Met Asn Ala Thr Asn His Ala Ile Xaa
 45 50
 5 Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa
 55 60
 Xaa Val Pro Lys Xaa Cys Cys Ala Pro Thr
 65 70
 Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 10 75 80
 Asp Xaa Ser Xaa Asn Val Ile Leu Xaa Lys
 85 90
 Xaa Arg Asn Met Val Val Xaa Ala Cys Gly
 95 100
 15 Cys His.
 and wherein Xaa at res. 2 = (Lys or Arg); Xaa at res. 3 = (Lys or Arg); Xaa at res. 11 = (Arg or
 Gln); Xaa at res. 16 = (Gln or Leu); Xaa at res. 19 = (Ile or Val); Xaa at res. 23 = (Glu or Gln);
 Xaa at res. 26 = (Ala or Ser); Xaa at res. 35 = (Ala or Ser); Xaa at res. 39 = (Asn or Asp); Xaa at
 res. 41 = (Tyr or Cys); Xaa at res. 50 = (Val or Leu); Xaa at res. 52 = (Ser or Thr); Xaa at res. 56
 20 = (Phe or Leu); Xaa at res. 57 = (Ile or Met); Xaa at res. 58 = (Asn or Lys); Xaa at res. 60 = (Glu,
 Asp or Asn); Xaa at res. 61 = (Thr, Ala or Val); Xaa at res. 65 = (Pro or Ala); Xaa at res. 71 =
 (Gln or Lys); Xaa at res. 73 = (Asn or Ser); Xaa at res. 75 = (Ile or Thr); Xaa at res. 80 = (Phe or

WO 95/33502

PCT/US95/06724

Tyr); Xaa at res. 82 = (Asp or Ser); Xaa at res. 84 = (Ser or Asn); Xaa at res. 89 = (Lys or Arg); Xaa at res. 91 = (Tyr or His); and Xaa at res. 97 = (Arg or Lys).

In still another preferred aspect, one or both of the polypeptide chain subunits of the osteogenically active dimer is encoded by nucleic acids which hybridize to DNA or RNA sequences encoding the active region of OP1 under stringent hybridization conditions. As used
5 herein, stringent hybridization conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

Given the foregoing amino acid and DNA sequence information, the level of skill in the art,
10 and the disclosures of numerous publications on osteogenic proteins, including U.S. Patent 5,011,691 and published PCT specification US 89/01469, published October 19, 1989, various DNAs can be constructed which encode at least the active domain of an osteogenic protein useful in the devices of this invention, and various analogs thereof (including species and allelic variants and those containing genetically engineered mutations), as well as fusion proteins, truncated forms
15 of the mature proteins, deletion and addition mutants, and similar constructs which can be used in the devices and methods of the invention. Moreover, DNA hybridization probes can be constructed from fragments of any of these proteins, or designed de novo from the generic sequence. These probes then can be used to screen different genomic and cDNA libraries to identify additional osteogenic proteins useful in the prosthetic devices of this invention.

20 The DNAs can be produced by those skilled in the art using well known DNA manipulation techniques involving genomic and cDNA isolation, construction of synthetic DNA from synthesized oligonucleotides, and cassette mutagenesis techniques. 15-100mer oligonucleotides may be synthesized on a DNA synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer. The DNA then may be electroeluted from
25 the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

The DNA from appropriately identified clones then can be isolated, subcloned (preferably into an expression vector), and sequenced. Plasmids containing sequences of interest then can be transfected into an appropriate host cell for protein expression and further characterization. The
30 host may be a procaryotic or eucaryotic cell since the former's inability to glycosylate protein will

WO 95/33502

PCT/US95/06724

not destroy the protein's morphogenic activity. Useful host cells include E. coli, Saccharomyces, the insect/baculovirus cell system, myeloma cells, CHO cells and various other mammalian cells. The vectors additionally may encode various sequences to promote correct expression of the recombinant protein, including transcription promoter and termination sequences, enhancer
5 sequences, preferred ribosome binding site sequences, preferred mRNA leader sequences, preferred signal sequences for protein secretion, and the like.

The DNA sequence encoding the gene of interest also may be manipulated to remove potentially inhibiting sequences or to minimize unwanted secondary structure formation. The recombinant osteogenic protein also may be expressed as a fusion protein. After being translated,
10 the protein may be purified from the cells themselves or recovered from the culture medium. All biologically active protein forms comprise dimeric species joined by disulfide bonds or otherwise associated, produced by folding and oxidizing one or more of the various recombinant polypeptide chains within an appropriate eucaryotic cell or in vitro after expression of individual subunits. A detailed description of osteogenic proteins expressed from recombinant DNA in E. coli and in
15 numerous different mammalian cells is disclosed in U.S. Patent No. 5,266,963, the disclosure of which is hereby incorporated by reference.

Alternatively, osteogenic polypeptide chains can be synthesized chemically using conventional peptide synthesis techniques well known to those having ordinary skill in the art. For example, the proteins may be synthesized intact or in parts on a solid phase peptide synthesizer,
20 using standard operating procedures. Completed chains then are deprotected and purified by HPLC (high pressure liquid chromatography). If the protein is synthesized in parts, the parts may be peptide bonded using standard methodologies to form the intact protein. In general, the manner in which the osteogenic proteins are made can be conventional and does not form a part of this invention.

25 Exemplification

The means for making and using the matrices and devices of the invention, as well as other material aspects concerning the nature and utility of these compositions, including how to make and how to use the subject matter claimed, will be further understood from the following, which constitutes the best mode currently contemplated for practicing the invention. It will be

WO 95/33502

PCT/US95/06724

appreciated that the invention is not limited to such exemplary work or to the specific details set forth in these examples.

In the exemplification, a hemi-joint reconstruction of an articulating synovial joint is resected into an existing joint locus. As will be appreciated by those having ordinary skill in the art, the methods and compositions of the invention equally can be applied to the formation of replacement body parts other than skeletal joints, as well as to skeletal joints other than articulating or synovial joints. Moreover, if desired, a replacement autogenous joint can be constructed in the recipient first by placing the device of the invention at another convenient locus distal to the defect site, for a time sufficient to induce formation of the replacement body part, and the autogenous body part thus formed then sutured into the joint locus for use.

Example 1. Reconstruction of a Mammalian Hemi-Joint

New Zealand white rabbits were used as the experimental model. Standard orthopedic surgical equipment and procedures were used.

As depicted in Fig. 2A, joint defects were created in a recipient by surgically resecting the entire gleno-humeral hemiarticular complex with the proximal two-thirds of the humerus. Allografts for implantation were prepared from hemi-joints excised from a donor animal with the articular surface of the glenohumeral joint. All allografts were extracted in ethanol and lyophilized using standard procedures, and as described herein above, to destroy the pathogenicity and antigenicity of the material. Specifically, intact joint complexes were excised, demarrowed and ethanol treated by exposure to 200ml-500ml of 200 proof ethanol for 72 hours at 40 C. Fresh ethanol was provided every 6-8 hours. Following ethanol treatment, the matrix was lyophilized and rehydrated in ethanol/TFA, with or without osteogenic protein. The treated hemi-joints comprised devitalized bone, articular cartilage, ligament, tendon, synovialcapsule and synovial membrane tissue.

As illustrated in Fig. 2B, all lyophilized, osteogenic protein-treated allografts were coated with OP-1 as described in U.S. 5,011,691. Specifically, mature, dimeric recombinant OP-1 (rhOP1) was solubilized in an acetonitrile trifluoro-acetic acid solution, combined with the lyophilized allograft, and implanted. 15-20 mg protein/8-10 g matrix, dry weight, was used. The distal bone portions of all allografts were secured in place with a four hole titanium miniplate. A

WO 95/33502

PCT/US95/06724

meticulous surgical reconstruction of the joint capsule was performed by suturing the lyophilized capsule ends to the endogenous capsule using standard surgical procedures well established in the art using standard surgical procedures well established in the art. This recreated an intact capsule and synovial lining, thereby restoring the synovial milieu of the grafted articular surface. Motion
5 was permitted almost immediately after surgery, again to restore normal joint conditions.

In some animals, local muscle flaps (cutaneous maximus muscle; Fig. 2C) were incorporated into the region of the defect by threading muscle into the marrow cavity of the allograft as depicted in Fig. 2D, using the method of Khouri as described in U.S. 5,067,963 the disclosure of which is incorporated herein by reference. Briefly, vascularized and convenient
10 muscle flaps were dissected using standard procedures well known to the practitioner in reconstructive surgery, so as to maintain a perfusing blood supply, and threaded inside the bone marrow cavities of the allografts.

Preliminary evaluations of the reconstructed hemi-joints were obtained by serial weekly radiographs using X-ray, and/or magnetic resonance imaging (MRI). Histological and mechanical
15 confirmatory evaluations were conducted upon sacrifice at 5 weeks and 6 months after surgery.

Mechanical evaluations involved standard range of motion (ROM) measurements obtained serially until sacrifice. Histological evaluations involved staining sagittal sections through the harvested allografts using standard techniques.

Briefly, identification of bona fide articular cartilage can be accomplished using
20 ultrastructural and/or biochemical parameters. For example, articular cartilage forms a continuous layer of cartilage tissue possessing identifiable zones. The superficial zone is characterized by chondrocytes having a flattened morphology and an extracellular network which does not stain, or stains poorly, with toluidine blue, indicating the relative absence of sulphated proteoglycans. Chondrocytes in the mid and deep zones have a spherical appearance and the matrix contains
25 abundant sulphated proteoglycans, as evidenced by staining with toluidine blue. Collagen fibers are present diffusely throughout the matrix. The chondrocytes possess abundant rough endoplasmic reticulum and are surrounded by extracellular network. The pericellular network contains numerous thin non-banded collagen fibers. The collagen in the interterritorial network is less compacted and embedded in electron translucent amorphous material, similar to articular

WO 95/33502

PCT/US95/06724

cartilage. Collagen fibers in the interterritorial region of the network exhibit the periodic banding characteristic of collagen fibers in the interterritorial zone of cartilage tissue.

Biochemically, the presence of Type II and Type IX collagen in the cartilage tissue is indicative of the differentiated phenotype of chondrocytes. The presence of Type II and/or Type IX collagen can be determined by standard gel electrophoresis, Western blot analysis and/or immunohisto-chemical staining using, for example, commercially available antibody. Other biochemical markers include hematoxylin, eosin, Goldner's Trichrome and Safranin-O.

Articular cartilage regeneration was evaluated histologically in the examples described herein using glycosaminoglycan-specific stains and techniques well-known in the art. For the initial histologic evaluation, the defect sites were bisected lengthwise through the center of the defect. The resulting halves and surrounding tissue were embedded in paraffin and sectioned across the center of the defect. One half of each defect was utilized for histological staining with toluidine blue and/or hematoxylin and eosin, Goldner's Trichrome and Safranin-O. The other half was used in preparing sections for immunostaining. Histological evaluations involved assessment of: glycosaminoglycan content in the repair cartilage; cartilage and chondrocyte morphology; and, structural integrity and morphology at the defect interface. The morphology of the repair cartilage was exhibited for the type of cartilage formed: articular vs. fibrotic by evaluating glycosaminoglycan content, degree of cartilage deposition, and the like.

Histological evaluations using standard methodologies well characterized in the art also allows assessment of new bone and bone marrow formation. See, for example, US Pat.No. 5,266,683, the disclosure of which is incorporated hereinabove by reference. Similarly, ligament and synovial capsule integrity can be monitored by MRI, as well as by histology upon sacrifice.

Example 2 Five Weeks Duration (Short Term)

For the 5 week study, four groups with 10 rabbits per group were implanted with lyophilized allografts. See Figs. 3A, 3B, 3C, and 3D. In Group 1, control lyophilized allograft free of osteogenic protein, was implanted (Fig. 3A). In Group 2, experimental lyophilized allograft was impregnated with OP-1 prior to implantation (Fig. 3B). In Group 3, control lyophilized allograft free of osteogenic protein, was implanted, with muscle flap threaded into marrow cavity (Fig. 3C). In Group 4, experimental lyophilized allograft was impregnated with OP-1

WO 95/33502

PCT/US95/06724

prior to implantation, and muscle flap 32 was threaded within the marrow cavity 33 (Fig. 3D).

As stated above, graft healing was followed non-invasively with serial X-rays and standard MRI (magnetic resonance imaging). By X-ray assessment, allografts treated with osteogenic protein had a noticeably thickened cortex by 1 week post-operative, as compared with control allografts (Groups 1, 3) which evidenced only a thin egg-shell-like cortex. By four weeks the majority control allografts had fractured and were unstable. In contrast, OP-1 treated allografts (Groups 2, 4) remained stable.

MRI also was used as a non-invasive means for following reformation of articular cartilage in the allografts. A dark signal produced by MRI represents absent or nonviable cartilage, while a bright signal indicates live, viable cartilage. Control allografts produced only a dark signal, when tested at 1, 3 and 5 weeks post-operative. These MRI findings were confirmed by histological analysis performed at 5 weeks post-operative. Sagittal sectioning through control allografts showed a degenerated articular surface with no live cells.

By contrast, the MRI findings of the articular caps from OP-1-treated allografts showed a bright signal by week 3 post-operative, indicating regeneration of viable articular cartilage. Histological analysis of the OP-1-treated allografts at week 5 revealed a layer of newly generated articular cartilage on top of the allograft matrix. The allografts of Group 4 showed somewhat thicker cartilage layers than those of Group 2, suggesting that the addition of the muscle flap may further enhance the rate of joint regeneration.

Additionally, joints regenerated with the OP-1-treated allografts regained near normal range of motion by the time they were harvested at 5 weeks post-reconstruction. The near normal range of motion also is indicative of the presence of lubricating synovial fluid. By contrast, the harvested control allografts were stiff and contracted at harvest. Thus, hemi-joint replacement devices of the invention succeeded in forming mechanically and functionally viable replacement joints, with an intact capsule, and synovium, and functioning ligament, bone and articular cartilage tissue. In the absence of osteogenic protein, the allografts, while not rejected by the donor, are insufficient on their own to generate a functional, weight bearing joint.

WO 95/33502

PCT/US95/06724

Example 3 Six Months Duration - (Long Term)

For the 6 month study, the variable of shaving off the old cartilaginous cap in the lyophilized allografts was introduced. Briefly, this was accomplished by mechanically sharing the articular cartilage cap of the joint surface.

5 The following groups were used with 4 rabbits per group: in Group 5, lyophilized allograft 34 with shaved articular surface, and muscle flap 32 were implanted (see Fig. 4A); in Group 6, control lyophilized allograft 30 with non-shaved articular surface, and muscle flap 32 were implanted (see Fig. 4B); in Group 7, lyophilized allograft 35 with shaved articular surface and OP-1, and muscle flap 36 treated with OP-1 were implanted (see Fig. 4C); and, in Group 8, lyophilized
10 allograft 37 with a non-shaved articular surface and OP-1, and muscle flap 36 treated with OP-1 were implanted (see Fig. 4D). Grafts in Groups 5-8 were harvested at 6 months after surgery.

Based upon pre-harvest imaging studies, the results collected by 3 months post-operative are consistent with the above-described results collected at 5 weeks. Intact allografts treated with OP-1 (Group 8) regenerated a live cartilaginous articular surface by 3 weeks when evaluated using
15 MRI. This articular cap is still present and even better developed at 3 months. Without OP-1 treatment of the allograft, (Group 6) there was negligible cartilage regeneration relative to the OP-1 treated groups.

Similarly, Group 8 rabbits (allograft + OP1, non-shaved) regained near normal range of motion (greater than 80%) in the reconstructed joint. Group 7 rabbits (allograft + OP1, shaved)
20 achieved only 50% range of motion, and Groups 5 and 6 (no OP1) achieved less than 30%.

As determined by histology, the devices of the invention were competent to induce and maintain both bone and articular cartilage formation in the appropriate context to one another in a long term study(greater than 6 months). Specifically, the rabbits of Group 8, demonstrated articular cartilage formation on the surface of bone, as evidenced morphologically by the presence
25 of resting, central and deeper zone chondrocytes. By contrast, in groups treated only with muscle flap, (Group 5 and 6) muscle was replaced with scar tissue. In the groups treated with shaved bone matrices, no significant cartilage regeneration was identified, demonstrating the requirement for cartilage-specific residues in articular cartilage formation in a non-vascularized milieu.

WO 95/33502

PCT/US95/06724

In both the short term and long term study, mechanically and functionally viable synovial joints resulted from the reconstructed hemijoints treated with osteogenic protein, as evidenced by morphology and biochemistry. In addition, new tissue formed, including articular cartilage, corresponding in shape, kind and structural relationship to the residues in the devitalized tissue which formed the matrix of the device. Collectively, these examples demonstrate that a device comprising osteogenic protein and an off-the-shelf, non-viable lyophilized, devitalized matrix can be transformed into a viable, mechanically and structurally functional replacement body part structure comprising plural distinct newly formed tissues which assume the shape and function of the original tissue. The device can restore normal function to a destroyed body part, including a destroyed skeletal joint, restoring mechanically and functionally viable plural distinct tissues, including bone and bone marrow, articular cartilage, ligament, tendon, synovial capsule and synovial membrane tissue. Moreover, these tissues are restored under substantially physiological conditions including, for example, from responding cells present in a synovial environment, and without exposure to avascularized muscle flap.

A device comprising osteogenic protein-treated matrices, including lyophilized allografts or xenografts as disclosed herein can lead to the formation of a new, mechanically, structurally and functionally viable replacement tissue, and to replacement body parts comprising plural distinct tissues, populated by the host cells, and without any of the limitations of prosthetic materials.

Those skilled in the art will know, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. These and all other equivalents are intended to be encompassed by the following claims.

WO 95/33502

PCT/US95/06724

5 SEQUENCE LISTING

(1) GENERAL INFORMATION:

- 10 (i) APPLICANT:
(A) NAME: CREATIVE BIOMOLCULES, INC
(B) STREET: 45 SOUTH STREET
(C) CITY: HOPKINTON
(D) STATE: MA
15 (E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 01748
(G) TELEPHONE: (508)-435-9001
(H) TELEFAX: (508)-435-0992
(I) TELEX:
- 20 (ii) TITLE OF INVENTION: MANUFACTURE OF AUTOGENOUS REPLACEMENT
BODY PARTS
- 25 (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: PATENT ADMINISTRATOR, TESTA, HURWITZ &
THIBEAULT
(B) STREET: 53 STATE STREET
30 (C) CITY: BOSTON
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02109
- 35 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
40 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
45 (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
- 50 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: KELLEY, ROBIN D.
(B) REGISTRATION NUMBER: 34,637
(C) REFERENCE/DOCKET NUMBER: CRP101PC
- 55 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/248-7000
(B) TELEFAX: 617/248-7100
- 60 (2) INFORMATION FOR SEQ ID NO:1:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1822 base pairs
(B) TYPE: nucleic acid
65 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA

WO 95/33502

PCT/US95/06724

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

10 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS

15 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "OP1"
 20 /evidence= EXPERIMENTAL
 /standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25 GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG 57
 Met His Val
 1

30 CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA 105
 Arg Ser Leu Arg Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
 5 10 15

35 CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC 153
 Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn
 20 25 30 35

40 GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG 201
 Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg
 40 45 50

45 CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC 249
 Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg
 55 60 65

CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG 297
 Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met
 70 75 80

50 CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC 345
 Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly
 85 90 95

55 GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC 393
 Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly
 100 105 110 115

60 CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC 441
 Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp
 120 125 130

65 ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC 489
 Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe
 135 140 145

CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC 537
 His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile

WO 95/33502

PCT/US95/06724

5	150	155	160	
	CCA GAA GGG GAA GCT GTC	ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC	585	
	Pro Glu Gly Glu Ala Val Thr	Ala Ala Glu Phe Arg Ile Tyr Lys Asp		
	165	170	175	
10	TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT	633		
	Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr			
	180	185	190	195
15	CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC	681		
	Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu			
	200	205	210	
20	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC	729		
	Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp			
	215	220	225	
25	ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG	777		
	Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu			
	230	235	240	
30	GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC	825		
	Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro			
	245	250	255	
35	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC	873		
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro			
	260	265	270	275
40	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC	921		
	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile			
	280	285	290	
45	CGG TCC ACG GGG AGC AAA CAG CGC AGC CAG AAC CGC TCC AAG ACG CCC	969		
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro			
	295	300	305	
50	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC	1017		
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser			
	310	315	320	
55	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065		
	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe			
	325	330	335	
60	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113		
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala			
	340	345	350	355
65	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161		
	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met			
	360	365	370	
70	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209		
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn			
	375	380	385	
75	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257		
	Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala			
	390	395	400	
80	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305		
	Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys			

WO 95/33502

PCT/US95/06724

```

5      405      410      415
TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC      1351
Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420      425      430
10 GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG      1411
GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG      1471
15 TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC      1531
ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC      1591
20 GCATAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT      1651
CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG      1711
GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC      1771
25 CTGTAATAAA TGTCACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A      1822

```

30 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

40 Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
   1           5           10           15
Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
   20           25           30
45 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
   35           40           45
50 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
   50           55           60
Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
   65           70           75           80
55 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
   85           90           95
Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
  100           105           110
60 Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
  115           120           125
65 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
  130           135           140
Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
  145           150           155           160

```

WO 95/33502

PCT/US95/06724

5 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
165 170 175

10 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
180 185 190

Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
195 200 205

15 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
210 215 220

Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
225 230 235 240

20 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
245 250 255

Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
260 265 270

Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
275 280 285

30 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
290 295 300

Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
305 310 315 320

35 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
325 330 335

Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
340 345 350

Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
355 360 365

45 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
370 375 380

Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
385 390 395 400

50 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
405 410 415

55 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
420 425 430

(2) INFORMATION FOR SEQ ID NO:3:

- 60 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- 65 (ii) MOLECULE TYPE: protein
- (ix) FEATURE:

WO 95/33502

PCT/US95/06724

5 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /label= OPX
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 10 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

15 Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1 5 10 15
 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 20 25 30
 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45
 25 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60
 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 65 70 75 80
 30 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95
 Xaa Ala Cys Gly Cys His
 100
 35

WO 95/33502

PCT/US95/06724

What is claimed is:

1. A device for implantation in a mammal which serves as a template to form in vivo a functional replacement body part comprising plural distinct tissues, the device comprising:
 - 5 a) a biocompatible, biodegradable matrix defining a structure which allows the attachment of infiltrating cells, comprising residues having specificity for, or derived from, said plural distinct tissues; and, having dimensions and shape which mimic that of the body part to be replaced; and disposed on the surface of said matrix,
 - 10 b) an osteogenic protein in an amount sufficient to induce formation of new said plural distinct tissues thereby to permit regeneration of tissues corresponding in shape and kind to said residues within a mammal.
2. The device of claim 1 wherein one of said plural distinct replacement tissues is a non-mineralized tissue.
3. The device of claim 1 wherein said matrix comprises devitalized tissue from a
15 mammalian donor.
4. The device of claim 1 wherein said matrix comprises residues of articular cartilage and of bone.
5. The device of claim 1 wherein said matrix comprises at least a portion of a skeletal joint.
- 20 6. The device of claim 1 or 5 wherein the matrix comprises dehydrated mammalian tissue.
7. The device of claim 2 wherein said replacement non-mineralized tissue is selected from the group consisting of articular cartilage, ligament, tendon, synovial capsule and synovial membrane tissue.
- 25 8. The device of claim 5 wherein said skeletal joint defines a synovial or articulating joint.

WO 95/33502

PCT/US95/06724

9. The device of claim 5 or 8 wherein said device defines a devitalized intact skeletal joint structure.
10. A device for implantation in a mammal forming in vivo articular cartilage replacement tissue in a skeletal joint, the device comprising:
- 5 osteogenic protein disposed on the surface of a biocompatible, bioresorbable matrix
- said matrix defining a structure which allows the attachment of infiltrating cells and which comprises residues specific for, or derived from, articular cartilage tissue.
11. A device for implantation in a mammal for forming in vivo replacement non-mineralized tissue in a skeletal joint, the device comprising:
- 10 osteogenic protein disposed on the surface of a biocompatible, bioresorbable matrix,
- said matrix defining a structure which allows the attachment of infiltrating cells and which comprises residues specific for, or derived from, non-mineralized skeletal joint
- 15 tissue corresponding in kind to said tissue to be replaced.
12. The device of claim 11 wherein said non-mineralized tissue is an avascular tissue.
13. The device of claim 10 wherein said non-mineralized tissue is selected from the group consisting of articular cartilage, ligament, synovial membrane and synovial capsule tissue.
- 20 14. The device of claim 10 or 11 wherein said matrix comprises devitalized allogenic or xenogenic tissue.
15. The device of claim 10 or 11 wherein said matrix comprises a material selected from the group consisting of: collagen, polymers comprising monomers of lactic acid, glycolic acid, butyric acid and combinations thereof, hydroxyapatite, tricalcium
- 25 phosphate, and mixtures thereof.

WO 95/33502

PCT/US95/06724

16. The device of claim 10 or 11 further comprising a material suitable for binding particulate matter to form a moldable solid.
17. The device of claim 1, 10 or 11 wherein said osteogenic protein comprises homodimers or heterodimers of OP-1, OP-2, BMP2, BMP3, BMP4, BMP5, BMP6, OPX, or functional equivalents thereof.
18. A method for inducing in a mammal the formation of an autologous replacement body part comprising plural distinct tissues, said method comprising the steps of:
- a) providing a device comprising osteogenic protein disposed on the surface of a bioresorbable, biocompatible matrix, said matrix defining a structure which allows the attachment of infiltrating cells, comprising residues having specificity for, or derived from, said plural distinct tissues, and having dimensions and shape which mimic that of the body part to be replaced; and
- b) implanting said device at a locus in a mammal, thereby to induce formation of tissues corresponding in shape and kind to said residues.
19. The method of claim 18 wherein said locus in said mammal defines an endogenous body part to be replaced.
20. The method of claim 18 wherein said matrix further comprises residues which are dimensioned to correspond in shape and structural relation to said plural distinct tissues to be replaced.
21. The method of claim 18 wherein the plural distinct tissues comprise bone and cartilage.
22. The method of claim 18 wherein said matrix comprises devitalized allogenic or xenogenic tissue.
23. The method of claim 18 wherein one of said plural distinct tissues is an avascular tissue.

WO 95/33502

PCT/US95/06724

24. A method for repairing in vivo articular cartilage on the surface of a bone, the method comprising the step of:
- 5 providing to said bone surface at a locus in a mammal a device comprising an osteogenic protein disposed on the surface of a biocompatible, bioresorbable matrix, said matrix comprising residues specific for, or derived from, cartilage, and defining a structure which allows the attachment of infiltrating cells.
25. The method of claim 24 wherein said locus occurs in a synovial cavity.
26. A method for restoring in a mammal a non-mineralized tissue in a skeletal joint, the method comprising the step of:
- 10 providing to said skeletal joint in a mammal a device comprising an osteogenic protein disposed on the surface of a biocompatible, bioresorbable matrix, said matrix comprising residues specific for, or derived from, tissue corresponding in kind to said non-mineralized tissue to be replaced, and defining a structure which allows the attachment of infiltrating cells.
- 15 27. The method of claim 26 wherein said non-mineralized tissue to be restored comprises avascular tissue.
28. The method of claim 26 wherein said non-mineralized tissue to be restored is selected from the group consisting of articular cartilage, tendon, ligament, synovial capsule and synovial membrane tissue.
- 20 29. The method of claim 24 or 26 wherein said matrix is derived from allogenic or xenogenic articular cartilage.
30. The method of claim 24 or 26 wherein said device comprises a moldable solid.
31. The method of claim 24 or 26 wherein said device comprises a flexible sheet.
- 25 32. The method of claim 24 or 26 wherein said device comprises collagen, polymers comprising lactic acid, butyric glycolic acid or mixtures thereof; hydroxyapatite and combinations thereof.

WO 95/33502

PCT/US95/06724

33. The method of claim 18, 24 or 26 wherein said osteogenic protein comprises homodimers or heterodimers of OP-1, OP-2, BMP2, BMP3, BMP4, BMP5, BMP6, OPX, or functional equivalents thereof.

WO 95/33502

PCT/US95/06724

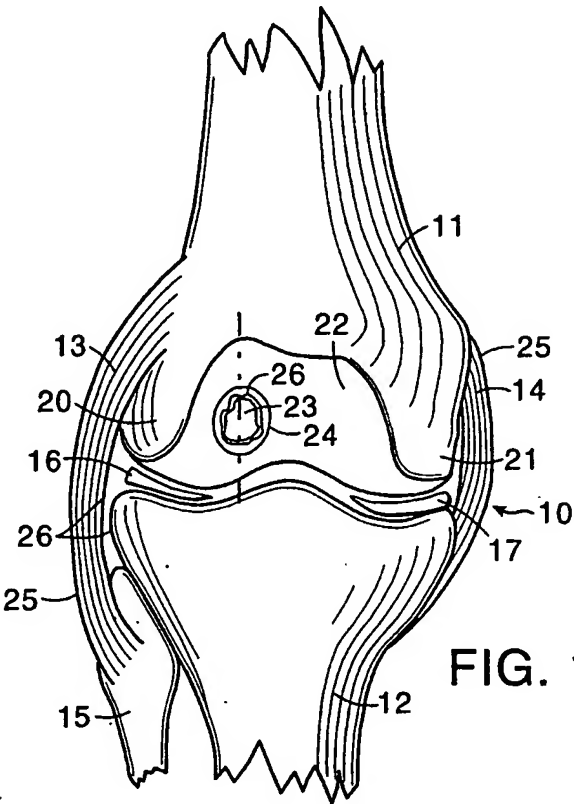


FIG. 1

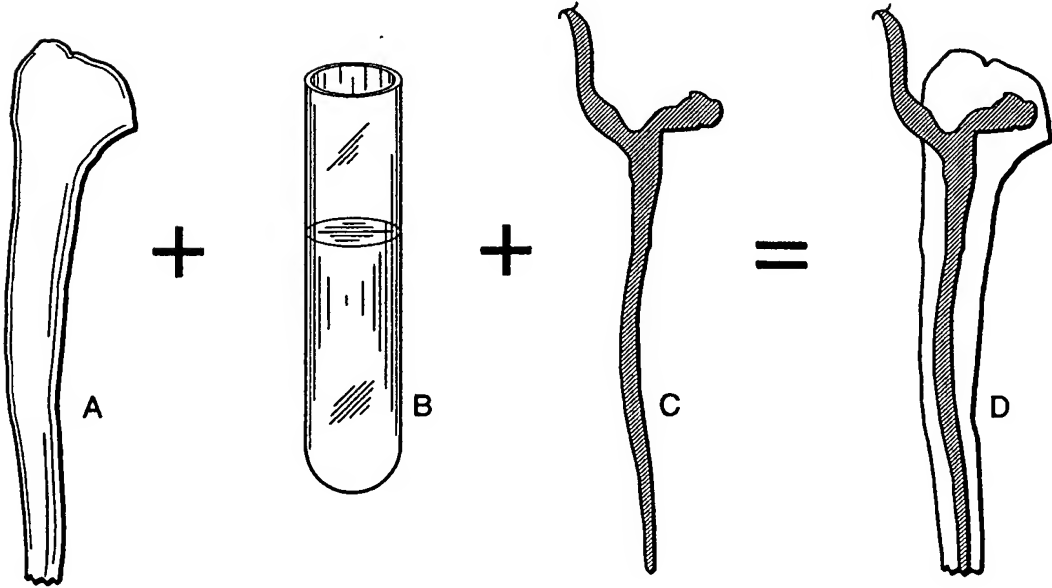


FIG. 2

1/3

WO 95/33502

PCT/US95/06724

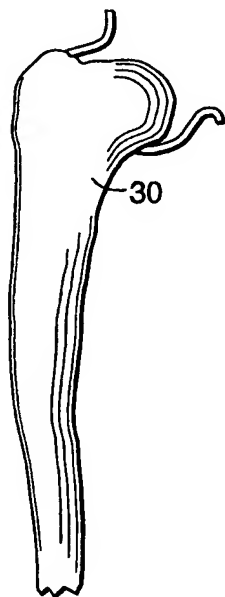


FIG. 3A

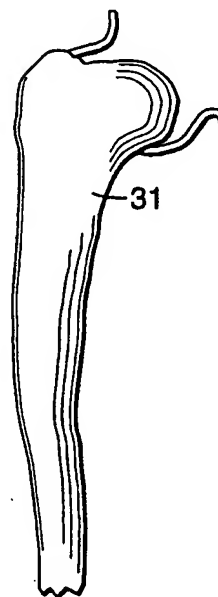


FIG. 3B

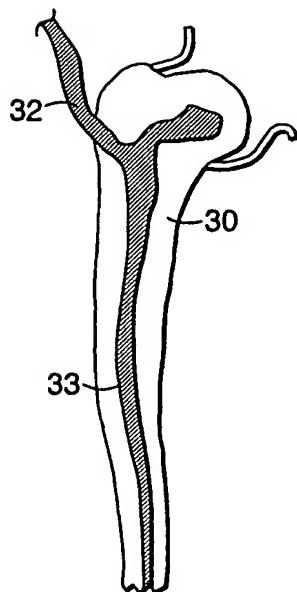


FIG. 3C

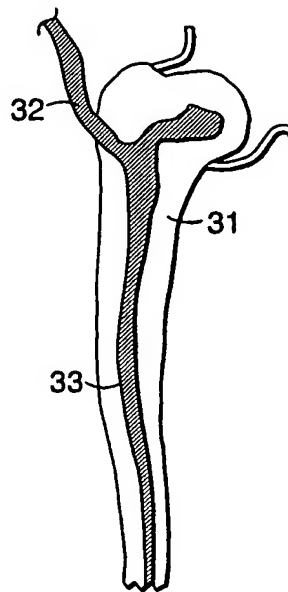


FIG. 3D

WO 95/33502

PCT/US95/06724

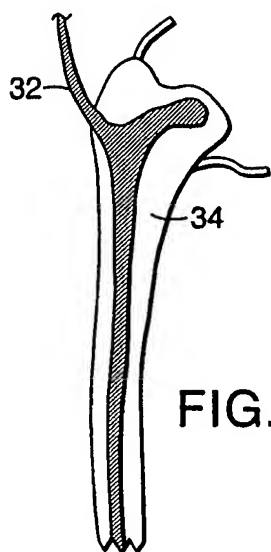


FIG. 4A

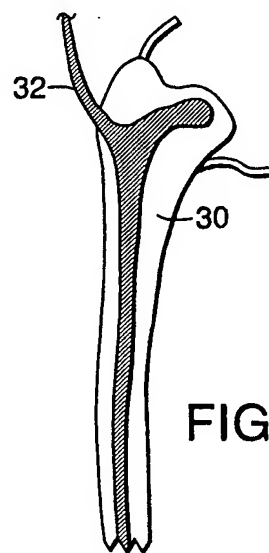


FIG. 4B

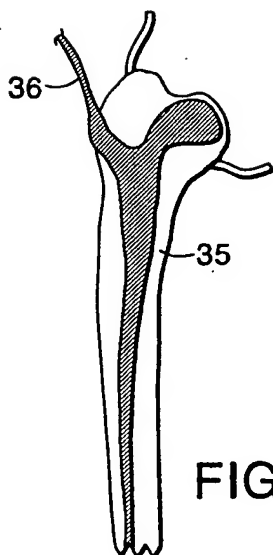


FIG. 4C

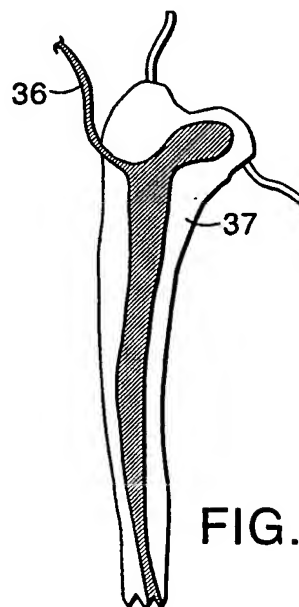


FIG. 4D

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/06724

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 567 391 (SQUIBB BRISTOL MYERS CO) 27 October 1993 see column 3, line 53 - line 59 see column 4, line 1 - line 5; claims ---	1-33
X	WO,A,93 25246 (STRYKER CORP) 23 December 1993 see claims; examples ---	1-33
X	EP,A,0 206 801 (NITTA GELATIN KK) 30 December 1986 see claims; examples ---	1
X	WO,A,91 18558 (CREATIVE BIOMOLECULES INC) 12 December 1991 cited in the application see claims ---	1
	--- -/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

27 September 1995

Date of mailing of the international search report

11.10.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

ESPINOSA, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06724

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,91 05802 (CREATIVE BIOMOLECULES INC) 2 May 1991 cited in the application see claims & US,A,5 266 683 ---	1
X	WO,A,89 09788 (CREATIVE BIOMOLECULES INC) 19 October 1989 cited in the application see claims & US,A,4 968 590 -----	1

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0567391	27-10-93	AU-B- 3699093	28-10-93
		CA-A- 2093836	25-10-93
		HU-A- 64863	28-03-94
		JP-A- 6025009	01-02-94
		NZ-A- 247421	27-09-94
		ZA-A- 9302661	26-11-93
WO-A-9325246	23-12-93	US-A- 5344654	06-09-94
		AU-B- 4599793	04-01-94
		CA-A- 2138270	23-12-93
		EP-A- 0646022	05-04-95
		JP-T- 7504680	25-05-95
EP-A-0206801	30-12-86	JP-B- 6062679	17-08-94
		JP-A- 62089629	24-04-87
		US-A- 4975527	04-12-90
WO-A-9118558	12-12-91	AU-B- 639574	29-07-93
		AU-A- 7961491	31-12-91
		EP-A- 0608211	03-08-94
		JP-T- 6505642	30-06-94
WO-A-9105802	02-05-91	US-A- 5171574	15-12-92
		AU-B- 648997	12-05-94
		AU-A- 6648190	16-05-91
		CA-A- 2042577	18-04-91
		EP-A- 0448704	02-10-91
		JP-T- 4502336	23-04-92
		US-A- 5266683	30-11-93
		US-A- 5344654	06-09-94
		US-A- 5354557	11-10-94
		CA-A- 2027259	18-04-91
WO-A-8909788	19-10-89	US-A- 4968590	06-11-90
		US-A- 5002770	26-03-91
		US-A- 5011691	30-04-91
		AU-B- 628050	10-09-92
		AU-B- 3444989	03-11-89
		AU-B- 618357	19-12-91
		AU-B- 3530589	03-11-89

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 95/06724

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-8909788		EP-A- 0372031	13-06-90
		EP-A- 0362367	11-04-90
		JP-T- 3500655	14-02-91
		JP-T- 3502579	13-06-91
		WO-A- 8909787	19-10-89
		US-A- 5108753	28-04-92
		US-A- 5182365	26-01-93
		US-A- 5250302	05-10-93
		US-A- 5266683	30-11-93
		US-A- 5344654	06-09-94
		US-A- 5324819	28-06-94
		US-A- 5354557	11-10-94
		US-A- 5258494	02-11-93
		AT-T- 124051	15-07-95
		AU-B- 627850	03-09-92
		AU-B- 5174790	26-09-90
		DE-D- 69020254	27-07-95
		EP-A- 0411105	06-02-91
		JP-T- 3504736	17-10-91
		WO-A- 9010018	07-09-90
		US-A- 4975526	04-12-90
		US-A- 5171574	15-12-92
		US-A- 5162114	10-11-92
